Limnologica **30** (2000) 162–168
http://www.urbanfischer.de/journals/limno

LIMNOLOGICA

Institut für Hydrobiologie und Fischereiwissenschaft, Universität Hamburg, Germany

Fatty Acid Composition of *Scenedesmus obliquus*: Correlation to Dilution Rates*

ALEXANDRA MAKULLA

With 2 Figures and 2 Tables

Key words: Growth rate, fatty acids, *Scenedesmus obliquus*, Chlorococcales, green algae

Abstract

To test the influence of growth rate on the pattern of fatty acids, *Scenedesmus obliquus* was exposed, in a chemostat culture, to different dilution rates, as these determine growth rates. Dilution rates varied from 0.0 to 0.48 d^{-1} (0.0 – 0.15 – 0.33 – 0.45 – 0.48 d^{-1}). In total, only few fatty acids showed a significant correlation to the dilution rate. However it seemed, that algae from the chemostat with zero flow-through differed more from steady state growing algae (i.e. positive dilution rates) in their patterns of fatty acids than within positive growth rates. Also, carbon and nitrogen were measured and C:N-ratios were calculated. The C:N-ratios support the grouping of the samples into two categories: "zero dilution rate" and "positive growth rates at steady state".

Introduction

In recent ecological studies on aquatic food webs, the question arose concerning whether food quality, as described by the pattern of fatty acids, is a parameter that can be used to describe the transfer of biomass into higher trophic levels (AHLGREN et al. 1990; MÜLLER-NAVARRA 1995a; GULATI & DEMOTT 1997). Fatty acids act not only as a source of energy, but also as constituents of membranes and precursors of hormones (URICH 1990). Especially linolenic acid (18:3ω3) and linoleic acid (18:2ω6) have a potential to be limiting resources for aquatic heterotrophic organisms (cf. review in BRETT & MÜLLER-NAVARRA 1997) because most animals fail to generate ω3-fatty acids *de novo*. Furthermore, EPA (eicosapentaenoic acid; 20:5ω3) and DHA (docosahexaenoic acid; 22:6ω3) are considered to be potentially hemiessential. To a certain degree, EPA and DHA can be sythesized from linolenic acid and linoleic acid (*Artemia* spp. fed rice bran; see ITO & SIMPSON 1996), but the majority has to be supplied by the food (BRETT & MÜLLER-NAVARRA 1997). This is the reason for the interest in the quality of food algae in experiments with herbivorous zooplankton.

Especially the food used in laboratory experiments should exhibit as little variation as possible in quality. However, the pattern of fatty acids in algae is known to be highly variable. First of all, the pattern depends on the systematic group the alga belongs to (LEE et al. 1971; MAYZAUD et al. 1976; MIYAZAKI 1983; VOLKMAN et al. 1989; AHLGREN et al. 1992; NAPOLITANO et al. 1996; LÉVEILLÉ et al. 1997). Secondly, the pattern of fatty acids varies according to the internal and external factors working on the algal cell (MORTENSEN et al. 1988; temperature, e.g. COHEN et al. 1988; AL-HASAN et al. 1991; THOMPSON et al. 1992; irradiance, e.g. THOMPSON et al. 1990; SUKENIK et al. 1989; nutrient limitation, e.g. PIORRECK et al. 1984; PIORRECK & POHL 1984, MÜLLER-NAVARRA 1995b). Furthermore, COHEN et al. (1988) and THOMPSON et al. (1992) concluded that part of the results they obtained was due to an influence of the growth rate or growth phase of the population. Therefore, the present study focusses on the relationships between growth rates and the pattern of fatty acids in the green alga *Scenedesmus obliquus* (formerly known as *S. acutus* MEYEN). This alga is widely used in experimental studies with herbivorous zooplankton, especially *Daphnia* spp. (e.g. LAMPERT 1977; PETERS & DEBERNARDI 1987; GLIWICZ 1990).

Material and Methods

A monoalgal culture of *Scenedesmus obliquus* (TURP.) KÜTZING (Chlorococcales, culture collection of algae, Göttingen: SAG strain 276-3a) was obtained from the Max-Planck-Institute for Limnology,

* This paper is dedicated to Prof. Dr. HARTMUT KAUSCH on the occasion of his 60th birthday.

Plön (Germany). Sterile techniques were used for algal cultures (stock cultures and experiments). WC medium (GUILLARD & LORENZEN 1972) with TES buffer instead of TRIS (pers. comm. S.S. KILHAM) was adjusted to a pH of 7.2 to 7.4 and sterile filtered using a Sartobran-P capsule (0.45/0.2 µm; SARTORIUS) and a liquid pump (ND 100 TT 23.18; KNF Neuberger). The sterilized medium was pumped into autoclaved bottles (Nalgene). A 600 ml vessel (Schmizo) provided with sterile filtered medium was inoculated with *S. obliquus*. This chemostat culture was exposed to a light intensity of about 100 µmol m^{-2} sec^{-1} (measured on the surface of the culture), a diurnal light cycle of 14:10 hours (light:dark) and a temperature of 20 °C. In a chemostat, the growth rate of the algal population accords with the daily dilution rate (NOVIK & SZILARD 1950; MONOD 1950).

After the algal culture had reached a steady state, samples were taken directly from the vessel (three hours after the onset of light). Sampling started with the dilution rate of 0.45 d^{-1} (only C:N data available) then the flow through was reduced to a dilution rate of 0.33 d^{-1}, and the algal culture was allowed to reach a steady state before the next sampling. The procedure for the next lower dilution rate was carried out accordingly. After samples for the dilution rate of 0.15 d^{-1} had been taken, the flow through was stopped, and the algae were harvested 48 h after the flow through had been stopped. Samples from a chemostat with a dilution rate of 0.48 d^{-1} were taken from another chemostat which had been set up with the conditions as described above (no data on C:N ratios available).

Subsamples of the sample from one dilution rate were filtered on precombusted and preweighed glassfibre filters (GF/C; Whatman) and stored under nitrogen in sealed caps at –20 °C until further processing. Prior to extraction or combustion, the filters were lyophilized (Christ BETA I) and weighed (Sartorius KC BA 100). C:N analysis was kindly done by H. STIBOR and T. HANSEN (Inst. Meereskunde Kiel, Germany). The dry weight of the samples used for the fatty acid analysis varied from 1.7 to 4.3 mg per filter. Fatty acids were extracted and methylated applying the methods of BLIGH & DYER (1959) and KATTNER & FRICKE (1986). An internal standard was added at the beginning of the extraction process (nonadecanoic acid, 19:0).

The fatty acid methyl esters (FAMEs) were detected in a gas chromatographer (Hewlett Packard 6890, with EPP) equipped with a PTV inlet, a fused-silica column (J&W; DB-225; 30 m length, 0.32 mm diameter, 0.25 µm film thickness) and a FID. Peaks were integrated with a HP software programm. Helium was used as carrier gas (constant velocity). Samples were injected splitless. Peaks were identified by comparison of the retention times with those of known FAMEs (e.g. standard mixtures S37, Supelco, and FAMEs purchased from Sigma). The concentrations of single fatty acids were calculated from the integrated areas of the identified FAMEs corrected for the response factor (*f*-factor; SCHOMBURG 1977), the internal standard and the filter blank. The amount of fatty acids per sample was then expressed in µg fatty acids per mg dry weight (DW) and also as a percentage of total fatty acids.

Results and Discussion

Dilution rates varied between 0.0 to 0.48 d^{-1}, which led to common growth rates when algae are used as convenient food in stock cultures and in experiments with daphnids (i.e. *Daphnia galeata,* e.g. MÜLLER-NAVARRA & LAMPERT 1996), because the cell density of the food will not need a lot of further concentration processes. Higher growth rates require a higher flow-through, i.e. more quantities of sterile medium.

Fig. 1 shows the correlation between dry weight and carbon or nitrogen content of the samples (per filter) and the resulting C:N ratios. C:N ratios decrease with increasing growth rates of *S. obliquus*; but, this trend is not statistically significant between growth rates 0.15 to 0.45 d^{-1}. The increase of the C:N-ratio of algae exhibiting a growth rate of zero indicates the beginning of a limitation by dissolved nutrients, e.g. nitrogen, as carbon is still available via aeration of the culture, and energy via illumination. Furthermore, a beginning limitation by phosphorus seems to be very likely at zero dilution rate. For nitrogen, the regression of the data without the sample from zero dilution rate gives a better fit than the fit with all data included (see Fig. 1): y = 0.0828x –

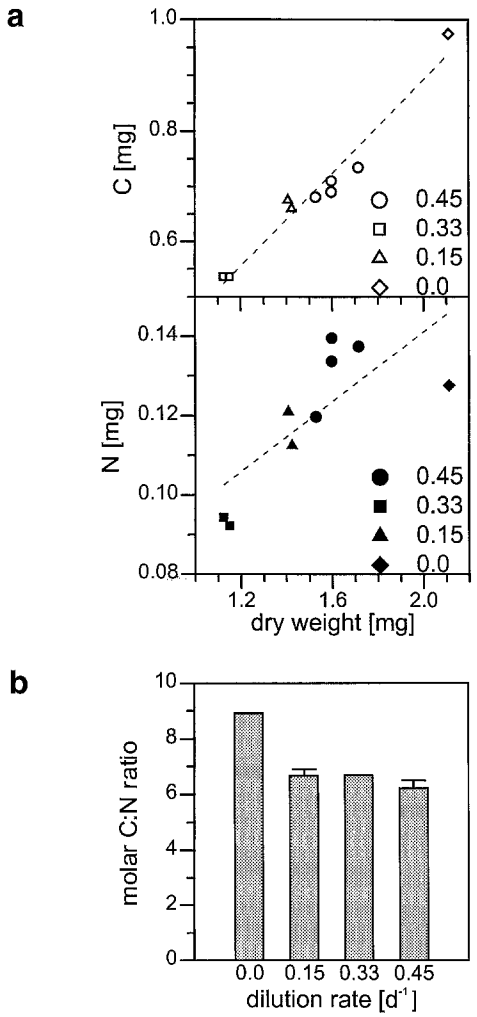

Fig. 1. a: Regression of particulate carbon (per filter) and nitrogen (per filter) versus dry weight (per filter) of *S. obliquus*; dilution rates are indicated with different symbols. Upper panel: y [mg C] = 0.4199x + 0.0510 (r^2 = 0.9596; P = 0.0000; n = 9); lower panel: y [mg N] = 0.0441x + 0.0529 (r^2 = 0.5727; P = 0.0182; n = 9). **b:** Molar C:N ratio at different dilution rates, given as mean with standard deviation.

0.0008 (r^2 = 0.9239; P = 0.0001; n = 8). For carbon, the significance does not differ: y = 0.3450x + 0.1547 (r^2 = 0.9472; P = 0.0000; n = 8). This is an indication, that the sample from zero dilution rate differs more from the samples with positive dilution rates than the samples within the group "positive dilution rates".

Table 1. Concentrations of fatty acids (FA) per biomass related to different dilution rates of *S. obliquus* (**a**). Relative amounts of FA [% of total FA] (**b**). The number of samples was two per dilution rate (0.0 – 0.15 – 0.33 d^{-1}) resp. three (0.48 d^{-1}). SAFA – saturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; USFA – unsaturated FA (USFA = MUFA + PUFA); n.d. – not detected. Values are given as means, with the standard deviation in parenthesis.

Table 1a.

	Dilution rate [d^{-1}]							
FA [µg mg DW^{-1}]	0		0.15		0.33		0.48	
12:0	n.d.		n.d.		n.d.		n.d.	
14:0	1.21	(0.33)	1.74	(0.01)	1.33	(0.05)	0.69	(0.03)
14:1ω5	n.d.		n.d.		0.52	(0.06)	0.08	(0.14)
16:0	12.51	(0.04)	9.38	(0.19)	9.17	(0.64)	11.28	(0.63)
16:1ω7	0.08	(0.11)	0.18	(0.02)	0.19	(0.03)	0.08	(0.07)
16:4ω1	n.d.		n.d.		n.d.		n.d.	
18:0	0.59	(0.01)	0.17	(0.00)	0.27	(0.05)	0.36	(0.17)
18:1ω9/12 t+c	9.38	(1.57)	3.00	(0.13)	3.33	(0.18)	3.78	(0.13)
18:1ω7	0.44	(0.02)	0.47	(0.03)	0.48	(0.09)	0.37	(0.02)
18:2ω6 t	1.73	(0.70)	1.46	(0.40)	1.23	(0.38)	1.63	(0.38)
18:2ω6	0.12	(0.17)	0.22	(0.08)	0.08	(0.11)	0.16	(0.17)
18:3ω6	0.07	(0.10)	0.24	(0.34)	0.47	(0.06)	0.26	(0.45)
18:3ω3	4.53	(2.67)	7.41	(3.63)	5.02	(3.19)	5.41	(0.33)
18:4ω3	0.96	(0.18)	0.98	(0.38)	0.70	(0.49)	0.77	(0.08)
20:0	n.d.		n.d.		n.d.		n.d.	
20:1ω9	0.40	(0.02)	0.21	(0.03)	0.21	(0.12)	0.12	(0.22)
20:2ω6	0.43	(0.61)	0.10	(0.14)	n.d.		n.d.	
20:3ω6	n.d.		n.d.		0.12	(0.17)	0.10	(0.18)
20:3ω3	n.d.		n.d.		0.08	(0.11)	n.d	
20:4ω6	0.08	(0.12)	0.06	(0.09)	0.22	(0.01)	0.15	(0.14)
20:5ω3	0.07	(0.10)	0.26	(0.36)	0.40	(0.05)	n.d.	
22:0	0.20	(0.28)	0.43	(0.01)	0.59	(0.07)	0.43	(0.11)
22:1ω9	0.11	(0.15)	n.d.		0.22	(0.06)	n.d.	
22:2ω6	n.d.		n.d.		n.d.		n.d.	
22:3ω3	n.d.		n.d.		n.d.		n.d.	
22:4ω6	n.d.		n.d.		n.d.		n.d.	
24:0	n.d.		0.29	(0.12)	0.28	(0.40)	0.54	(0.21)
22:6ω3	n.d.		n.d.		n.d.		n.d.	
24:1ω9	n.d.		n.d.		0.08	(0.11)	n.d.	
Σ FA	32.91	(6.60)	26.60	(4.65)	24.99	(5.36)	26.21	(0.41)
Σ ω3	5.56	(2.96)	8.65	(4.38)	6.20	(3.75)	6.18	(0.28)
Σ ω6	2.43	(1.69)	2.08	(0.78)	2.12	(0.72)	2.30	(0.41)
ω3 : ω6	2.46	(0.49)	4.04	(0.58)	2.79	(0.83)	2.74	(0.43)
ω6 : ω3	0.41	(0.08)	0.25	(0.04)	0.38	(0.11)	0.37	(0.05)
SAFA	14.51	(0.09)	12.00	(0.30)	11.64	(0.96)	13.29	(0.41)
MUFA	10.41	(1.87)	3.87	(0.21)	5.03	(0.07)	y4.44	(0.19)
PUFA	7.98	(4.64)	10.73	(5.16)	8.32	(4.46)	8.48	(0.66)
USFA : SAFA	1.27	(0.44)	1.22	(0.44)	1.13	(0.28)	0.97	(0.09)
SAFA : MUFA	1.42	(0.25)	3.11	(0.09)	2.32	(0.22)	3.00	(0.22)
SAFA : PUFA	2.18	(1.26)	1.27	(0.64)	1.60	(0.74)	1.58	(0.17)
MUFA : PUFA	1.49	(0.63)	0.41	(0.22)	0.71	(0.39)	0.53	(0.03)
MUFA + PUFA	18.40	(6.52)	14.60	(4.95)	13.34	(4.39)	12.92	(0.81)
PUFA : SAFA	0.55	(0.32)	0.90	(0.45)	0.70	(0.33)	0.64	(0.07)
PUFA : Σ FA	0.23	(0.09)	0.39	(0.13)	0.32	(0.11)	0.32	(0.02)

Table 1 shows the identified fatty acids for different growth rates of *S. obliquus* expressed as [µg mg DW^{-1}] (Table 1a) and as [% of total fatty acids] (Table 1b). Only those peaks are listed which could be identified beyond doubt. The pattern of fatty acids in *S. obliquus* is very typical for Chlorococcales (e.g. AHLGREN et al. 1992): it is very rich in palmitic acid (16:0), linolenic acid (18:3ω3), 18:1ω9/12 (18:1ω9: oleic acid), but very poor in EPA. DHA was not detected in any of the samples. Fig. 2 suggests that the concentration of fatty acids per dry weight decreases with increasing growth rate, however, this trend is not statistically significant. If all data are taken into account, the regression is $y = 30.7544 - 12.1536x$ ($r^2 = 0.2698$; $P = 0.1518$; $n = 9$). This trend is comparable with the effect of nutrient limitation on the fatty acid profile (PIORRECK et al. 1984; PIORRECK & POHL 1984; MÜLLER-NAVARRA 1995b), i.e. the concentration or percentage of fatty acids in nutrient limited cells increases. Often, even in experimental studies, it is practically impossible to distinguish between the effects of growth rate and those of nutrient limitation when cultures are not grown in steady state conditions. The slower the growth rate, the more likely resource limitation can occur.

Table 2 shows that most of the trends between fatty acids or groups of fatty acids are not statistically significant. If all data are included, only 18:1ω9/12 and MUFA (monounsaturated fatty acids) decrease significantly with increasing growth rate. On the other hand, within the samples of the group "positive dilution rates", myristic acid (14:0) decreas-

Table 1b.

	Dilution rate [d^{-1}]							
FA [% Σ FA]	0		0.15		0.33		0.48	
12:0	n.d.		n.d.		n.d.		n.d.	
14:0	3.64	(0.29)	6.64	(1.19)	5.46	(1.39)	2.64	(0.11)
14:1ω5	n.d.		n.d.		2.14	(0.72)	0.30	(0.52)
16:0	38.78	(7.65)	35.86	(6.97)	37.29	(5.45)	43.06	(3.05)
16:1ω7	0.22	(0.30)	0.70	(0.21)	0.79	(0.28)	0.31	(0.27)
16:4ω1	n.d.		n.d.		n.d.		n.d.	
18:0	1.84	(0.39)	0.64	(0.10)	1.09	(0.05)	1.36	(0.64)
18:1ω9/12 t+c	28.60	(0.97)	11.51	(2.51)	13.57	(2.19)	14.44	(0.55)
18:1ω7	1.36	(0.22)	1.81	(0.43)	2.01	(0.80)	1.42	(0.10)
18:2ω6 t	5.14	(1.08)	5.44	(0.55)	4.85	(0.50)	6.21	(1.47)
18:2ω6	0.31	(0.44)	0.81	(0.17)	0.27	(0.38)	0.61	(0.63)
18:3ω6	0.18	(0.25)	0.81	(1.15)	1.90	(0.16)	0.97	(1.67)
18:3ω3	13.21	(5.48)	27.08	(8.93)	19.15	(8.67)	20.64	(0.98)
18:4ω3	2.91	(0.03)	3.62	(0.81)	2.65	(1.40)	2.93	(0.37)
20:1ω9	1.25	(0.20)	0.80	(0.25)	0.90	(0.68)	0.47	(0.81)
20:2ω6	1.15	(1.63)	0.41	(0.58)	n.d.		n.d.	
20:3ω6	n.d.		n.d.		0.41	(0.58)	0.39	(0.68)
20:3ω3	n.d.		n.d.		0.28	(0.39)	n.d.	
20:4ω6	0.22	(0.31)	0.22	(0.31)	0.92	(0.22)	0.58	(0.53)
20:5ω3	0.19	(0.27)	0.86	(1.21)	1.66	(0.55)	n.d.	
22:0	0.71	(1.01)	1.62	(0.23)	2.44	(0.78)	1.63	(0.41)
22:1ω9	0.29	(0.41)	n.d.		0.94	(0.43)	n.d.	
22:2ω6	n.d.		n.d.		n.d.		n.d.	
22:3ω3	n.d.		n.d.		n.d.		n.d.	
22:4ω6	n.d.		n.d.		n.d.		n.d.	
24:0	n.d.		1.16	(0.67)	0.99	(1.40)	2.05	(0.78)
22:6ω3	n.d.		n.d.		n.d.		n.d.	
24:1ω9	n.d.		n.d.		0.28	(0.39)	n.d.	
Σ FA	100		100		100		100	
Σ ω3	16.31	(5.72)	31.56	(10.95)	23.75	(9.90)	23.57	(0.79)
Σ ω6	7.00	(3.72)	7.69	(1.60)	8.36	(1.07)	8.76	(1.44)
SAFA	44.97	(8.76)	45.93	(9.16)	47.27	(6.27)	50.73	(2.34)
MUFA	31.72	(0.67)	14.83	(3.40)	20.63	(4.71)	16.94	(0.54)
PUFA	23.31	(9.43)	39.25	(12.55)	32.10	(10.98)	32.33	(2.03)
MUFA + PUFA	55.03	(8.76)	54.07	(9.16)	52.73	(6.27)	49.27	(2.34)

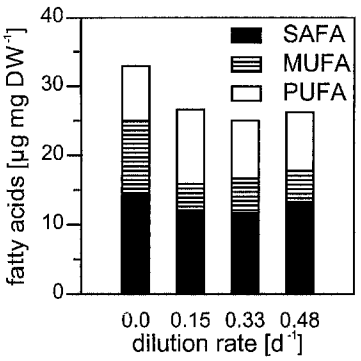

Fig. 2. Concentration of total fatty acids per biomass as a sum of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in *S. obliquus* depending on the dilution rate. The number of samples was two per dilution rate (0.0 – 0.15 – 0.33 d^{-1}) resp. three (0.48 d^{-1}).

es and palmitic acid increases with increasing growth rate. Also, in terms of the percentage of total fatty acids, the dominance of palmitic acid increases with increasing growth rate ($y = 31.7104 + 22.2904x$; $r^2 = 0.3812$; $P = 0.1396$; $n = 7$). SUKENIK et al. (1989) found an increase of the percentage of palmitic acid when cultures were grown under light-saturated conditions. The recalculated data of MÜLLER-NAVARRA (1995b) show the same trend for phosphorus-saturated *S. acutus*, but not for *Cyclotella meneghiniana:* the percentage of palmitic acid is 24% in phosphorus-limited and 33% in phosphorus-rich *S. acutus* (now *S. obliquus*); at the same time phosphorus-limited cells are richer in palmitic acid per carbon. Contrary to the present study, PIORRECK et al. (1984) found a tendency for the percentage of palmitic acid to decrease with increasing nitrogen availability (for nitrogen-limited *S. obliquus*).

In *S. obliquus*, the ratio of unsaturated:saturated fatty acids decreased with increasing dilution rate (Tables 1 and 2). However, this trend is not statistically significant. Also, the tendency for a decreasing concentration per dry weight of unsaturated fatty acids (MUFA + PUFA; mono- and polyunsaturated fatty acids) with increasing growth rate is not significant. THOMPSON et al. (1992) described the ratio of unsaturated:saturated fatty acids as a function of growth rate, but only for three out of eight investigated species of marine algae.

In experiments on the effect of resource limitation on the pattern of fatty acids, COHEN et al. (1988) deduced that a higher growth rate promoted higher concentrations of EPA (expressed as % of total fatty acids). They correlated especially the growth rate to the R ratio (R = 20:4ω6 [%] : EPA [%]; COHEN et al. 1988). The effects they found were strong: the relation between both fatty acids switched with increasing growth rates. On the other hand, the experiments were designed to find effects of temperature and light intensity on the pattern of fatty acids, so that not only the growth rates of the algae in the experiments varied. COHEN et al. (1988) investigated *Porphyridium cruentum* (Rhodophyceae) which is rich in arachidonic acid and EPA. *S. obliquus* is poor in the latter PUFA, but rich in linolenic acid. Here, in *S. obliquus*, with increasing growth rate, the concentration per dry weight of linolenic acid fluctuated over the observed dilution rates (Tables 1 and 2). Also, within the fraction of both ω3 and ω6 fatty acids, there is no uniform trend, but, corresponding to AHLGREN et al. (1992), it is ≥2. The ratio of ω6:ω3 fatty

Table 2. Linear regressions of single fatty acids (FA) and groups of FA as a function of the dilution rate in *S. obliquus*, calculated for the concentrations of fatty acids per biomass. SAFA – saturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; USFA – unsaturated FA (USFA = MUFA + PUFA).

All data included (n = 9)			
		r^2	P
14:0	y = 1.5518 – 1.3962x	0.4078	0.0642
16:0	y =11.2495 – 3.9058x	0.1261	0.3140
18:1ω9/12t+c	y = 7.1810 – 9.1037x	0.4464	0.0492
18:2ω6	y = 0.1467 – 0.0020x	0.0000	0.9934
18:3ω3	y = 5.6093 – 0.1394x	0.0002	0.9750
20:5ω3	y = 0.2024 – 0.1516x	0.0195	0.7204
Σ ω3	y = 6.7988 – 0.7678x	0.0035	0.8803
Σ ω6	y = 2.2728 – 0.1260x	0.0011	0.9320
ω3:ω6	y = 3.1055 – 0.4249x	0.0156	0.7490
ω6:ω3	y = 0.3501 + 0.0178x	0.0017	0.9152
Σ FA	y = 30.7544 – 12.1536x	0.2698	0.1518
SAFA	y = 13.3836 – 1.7810x	0.0844	0.4482
MUFA	y = 8.2993 – 9.4798x	0.4670	0.0424
PUFA	y = 9.0716 – 0.8958x	0.0032	0.8850
MUFA + PUFA	y = 17.3708 – 10.3755x	0.2640	0.1571
USFA : SAFA	y = 1.2940 – 0.6162x	0.1938	0.2357
Only data of "positive dilution rates" (n = 7)			
		r^2	P
14:0	y = 2.2699 – 3.2029x	0.9698	0.0001
16:0	y = 8.0758 + 6.0001x	0.5795	0.0468
18:1ω9/12t+c	y = 2.61529 + 2.3823x	0.8869	0.0015
18:2ω6	y = 0.3133 + 0.0021	0.0000	0.9983
18:3ω3	y = 7.8601 – 5.8018x	0.1450	0.3994
20:5ω3	y = 0.47292 – 0.8320x	0.2675	0.2346
Σ ω3	y = 9.3834 – 7.2719x	0.1649	0.3660
Σ ω6	y = 1.9530 + 0.6786x	0.0392	0.6703
ω3:ω6	y = 4.4485 – 3.8611x	0.5192	0.0677
ω6:ω3	y = 0.2154 + 0.3565x	0.4048	0.1245
Σ FA	y = 26.2836 – 0.9062x	0.0020	0.9239
SAFA	y = 11.0442 + 4.1072x	0.4276	0.1111
MUFA	y = 3.9031 + 1.5799x	0.2209	0.2872
PUFA	y = 11.3363 – 6.5933x	0.1033	0.4820
MUFA + PUFA	y = 15.2394 – 5.0134x	0.0679	0.5726
USFA : SAFA	y = 1.3509 - 0.7594x	0.2033	0.3099

acids, which can be compared to the R (explanation see above) of COHEN et al. (1988), does not show a comparable correlation with the data of COHEN et al. (1988; see Fig. 2), but it cannot be predicted what will happen to the pattern of fatty acids in *S. obliquus* when dilution rates resp. growth rates are higher.

Whatever the different growth rates, the EPA (expressed as µg mg DW^{-1}) does not exhibit a tendency to increase or decrease. This fatty acid is of special interest because MÜLLER-NAVARRA (1995a) found a strong correlation between the concentration of EPA in lake seston and the growth rate of *Daphnia galeata*. Where experimental work with herbivorus zooplankton is concerned, one must consider that the food quality of *S. obliquus* is low at any of the observed growth rates, with respect to EPA. But, the essential fatty acids linolenic and linoleic acid, which can be further processed to EPA, do not show a significant correlation to the dilution rate.

The results of the present study and the data in the literature reflect that there are no uniform trends in the relationship between growth rate and the pattern of fatty acids. Some fatty acids seem to increase with increasing growth rates, others decrease. Only a few regressions turned out to be significant, most trends are not statistically significant. However, it seems to make sense to group the data into two categories: "zero dilution rate" and "positive growth rates at steady state". It can not be assumed that the algae grown in the chemostat where the flow-through had been stopped were not growing any more. But certainly they were no longer in a state of equilibrium with the resources. Therefore, the effects of resource limitation, i.e. limitation of nutrients and light, on the pattern of fatty acids in algae can be stronger than the effects of the growth rate.

For experimental studies with herbivorous zooplankton, it is recommended to keep the growth conditions for food algae as constant as possible i.e. to achive a steady state which can be checked by the dilution rates and/or growth rates. In a chemostat, algae can be grown at steady state and high food densities can be achieved when dilution rates are moderately. Within the observed dilution rates, the influence of growth rates on the pattern of fatty acids was only weak in most cases. Yet the question remains unanswered, what will happen to the pattern of fatty acids when the dilution rates are higher than those described in this study.

Acknowledgements: Financial support was given by the Deutsche Forschungsgemeinschaft, project KA 292/24. The use of a gaschromatographer by the AK Dr. U. BROCKMANN, University of Hamburg, and by the team of Prof. Dr. C. R. GOLDMAN, UC Davis/U.S.A. is greatly acknowledged. The author greatly appreciates the measuring of C and N by Dr. HERWIG STIBOR and THOMAS HANSEN, Inst. Meereskunde, University of Kiel. The manuscript benefited from the comments of D.C. MÜLLER-NAVARRA and two anonymous reviewers. An earlier version was improved liguistically by CAROL BERGER.

References

AHLGREN, G., LUNDSTEDT, L., BRETT, M. & FORSBERG, C. (1990): Lipid composition and food quality of some freshwater microalgae. J. Plankton Res. **12**: 809–818.

– GUSTAFSSON, I.-B. & BOBERG, M. (1992): Fatty acid content and chemical composition of freshwater microalgae. J. Phycol. **28**: 37–50.

AL-HASAN, R., HANTASH, F.M. & RADWAN, S.S. (1991): Enriching marine macroalgae with eicosatetraenoic (arachidonic) and eicosapentaenoic acids by chilling. Appl. Microbiol. Biotechnol. **35**: 530–535.

BLIGH, E.G. & DYER, W.J (1959): A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**: 911–917.

BRETT, M.T. & MÜLLER-NAVARRA, D.C. (1997): The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biol. **38**: 483–499.

COHEN, Z., VONSHAK, A. & RICHMOND, A. (1988): Effect of environmental conditions on fatty acid composition of the red alga *Porphyridium cruentum*: correlation to growth rate. J. Phycol. **24**: 328–332.

GLIWICZ, Z.M. (1990): Food thresholds and body size in cladocerans. Nature **343**: 638–640.

GUILLARD, R.R.L. & LORENZEN, C.L. (1972): Yellow-green algae with chlorophyllide *c*. J. Phycol. **8**: 10–14.

GULATI, R.D. & DEMOTT, W. [eds.] (1997): The role of food quality for zooplankton. Freshwater Biol. **38**.

ITO, M.K. & SIMPSON, K.L. (1996): The biosynthesis of ω3 fatty acids from 18:2ω6 in *Artemia* spp. Comp. Biochem. Pysiol. **115B**: 69–76.

KATTNER, G. & FRICKE, H.S. (1986): Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J. Chromatogr. **361**: 263–286.

LAMPERT, W. (1977): Studies on the carbon balance of *Daphnia pulex* DE GEER as related to environmental conditions. Arch. Hydrobiol./Suppl. **48** (Falkau-Arbeiten **10**): 287–309.

LEE, R.F., NEVENZEL, J.C. & PFAFFENHÖFER, G.A. (1971): Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar. Biol. **9**: 99–108.

LÉVEILLÉ, J.-C., AMBLARD, C. & BOURDIER, G. (1997): Fatty acids as specific algal markers in a natural lacustrian phytoplankton. J. Plankton Res. **19**: 469–490.

MAYZAUD, P., EATON, C.A. & ACKMAN, R.G. (1976): The occurrence and distribution of octadecapentaenoic acid in a natural plankton population. A possible food chain index. Lipids **11**: 858–862.

MIYAZAKI, T. (1983): Compositional changes of fatty acids in particulate matter and water temperature, and their implications to the seasonal succession of phytoplankton in a hypertrophic lake, Lake Kasumigaura, Japan. Arch. Hydrobiol. **99**: 1–14.

MONOD, J. (1950): La technique de culture continue. Theorie et applications. Ann. Inst. Pasteur **79**: 390–410.

MORTENSEN, S.H., BORSHEIM, K.Y., RAINUZZO, J.R. & KNUTSEN, G. (1988): Fatty acid and elemental composition of the marine diatom *Chaetoceros gracilis* SCHÜTT. Effects of silicate deprivation, temperature and light intensity. J. Exp. Mar. Biol. Ecol. **122**: 173–185.

MÜLLER-NAVARRA, D. (1995a): Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. Arch. Hydrobiol. **132**: 297–307.

– (1995b): Biochemical versus mineral limitation in *Daphnia*. Limnol. Oceanogr. **40**: 1209–1214.
– & LAMPERT, W. (1996): Seasonal patterns of food limitation in *Daphnia galeata*: seperating food quantitiy and food quality effects. J. Plankton Res. **18**: 1137–1157.
NAPOLITANO, G.E., SHANTA, N.C., HILL, W.R. & LUTTRELL, A.E. (1996): Lipid and fatty acid compositions of stream periphyton and Stoneroller Minnows (*Campostoma anomalum*): trophic and environmental implications. Arch. Hydrobiol. **137**: 211–225.
NOVIK, A. & SZILARD, L. (1950): Description of a chemostat. Science **112**: 715–716.
PETERS, R.H. & DE BERNARDI, R. [eds.] (1987): Daphnia. Memoire dell'Instituto Italiano di Idrobiologia **45**.
PIORRECK, M., BAASCH, K.-H. & POHL, P. (1984): Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry **23**: 207–216.
– & POHL, P. (1984): Formation of biomass, total protein, chlorophylls, lipids and fatty acids in green and blue-green algae during one growth phase. Phytochemistry **23**: 217–223.
SCHOMBURG, G. (1977): Gaschromatographie. Verlag Chemie.
SUKENIK, A., CARMELI, Y. & BERNER, T. (1989): Regulation of fatty acid composition by irradiance level in the eustigmatophyte *Nannochloris* sp. J. Phycol. **25**: 686–692.
THOMPSON, P.A., HARRISON, P.J. & WHYTE, J.N.C. (1990): Influence of irradiance on the fatty acid composition of phytoplankton. J. Phycol. **26**: 278–288.
– GUO, M.-X., HARRISON, P.J. & WHYTE, J.N.C. (1992): Effects of variation in temperature. II. On the fatty acid composition of eight species of marine phytoplankton. J. Phycol. **28**: 488–497.
URICH, K. (1990): Vergleichende Biochemie der Tiere. Fischer.
VOLKMAN, J.K., JEFFREY, S.W., NICHOLS, P.D., ROGERS, G.I. & GARLAND, C.D. (1989): Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. **128**: 219–240.

Author's address: Dr. ALEXANDRA MAKULLA, Universität Hamburg, Institut für Hydrobiologie und Fischereiwissenschaft, Hydrobiologische Abteilung, Zeiseweg 9, D - 22765 Hamburg, Germany.